ARBOVIRUS SEROLOGICAL SURVEY OF INDONESIANS RESIDING WITHIN A 50 Km. RADIUS OF JAKARTA IN 1963

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Naval Medical Research Unit No. 2

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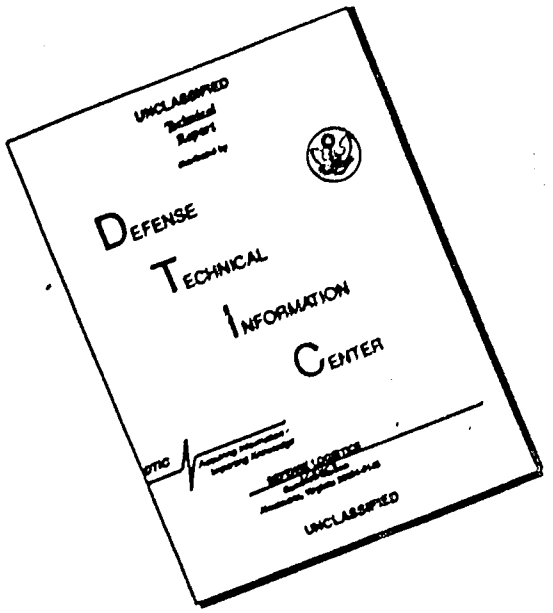

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AN ARBOVIRUS SERUM SURVEY WAS CONDUCTED IN MARCH 1963 AMONG RESIDENTS OF VARIOUS COMMUNITIES WITHIN A 50 KM RADIUS OF JAKARTA, INDONESIA. THE 497 SERA COLLECTED WERE ANALYZED BOTH QUALITATIVELY AND QUANTITATIVELY BY TCNT FOR ANTIBODIES TO CHIKUNGUNYA, SINDBIS, EASTERN EQUINE ENCEPHALITIS, JAPANESE ENCEPHALITIS, YELLOW FEVER, AND BUNYAMWERA VIRUSES. EVIDENCE FOR THE PRESENCE OF GROUP A, B AND BUNYAMWERA VIRUSES IN THE AREAS SURVEYED IS PRESENTED. ANALYSIS OF THE DATA OBTAINED SUGGESTS THAT CHIKUNGUNYA, JAPANESE ENCEPHALITIS AND A VIRUS ANTIGENICALLY RELATED TO BUNYAMWERA WERE MOST LIKELY PRESENT IN MOST OF THESE COMMUNITIES.

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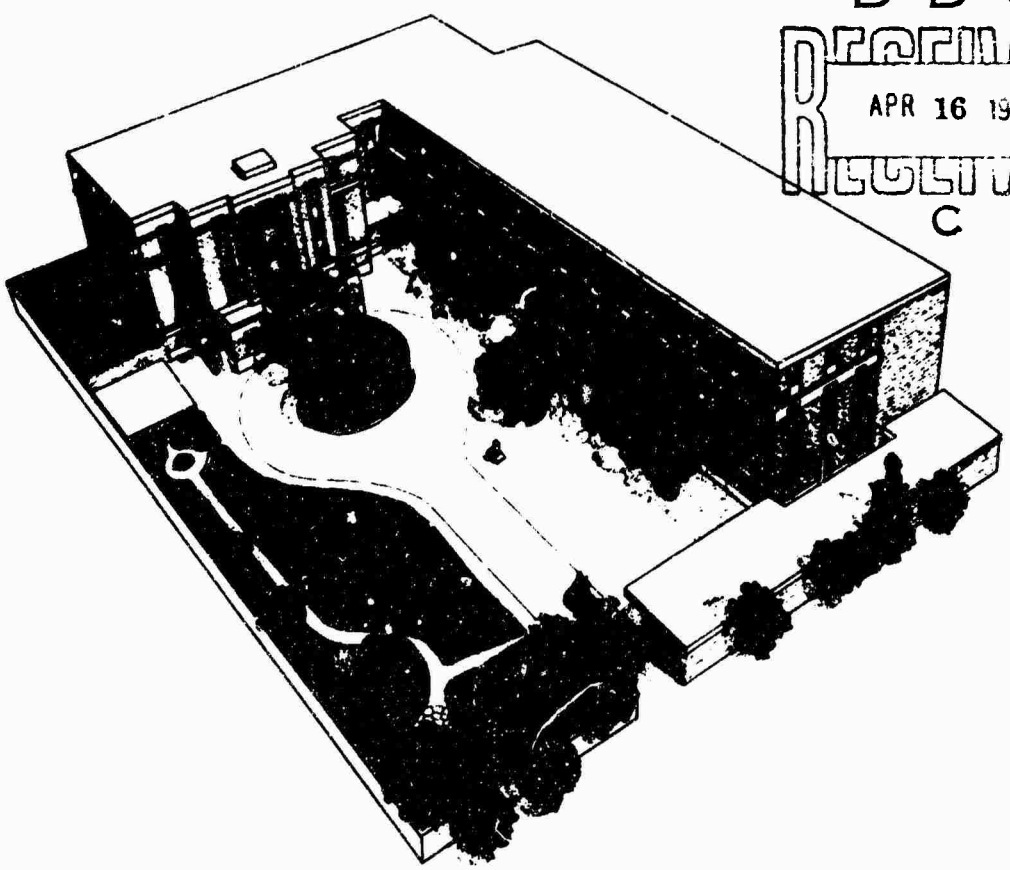

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ADMINISTRATIVE INFORMATION

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Arbovirus Serological Survey of Indonesians Residing Within a 50 Km. Radius of Jakarta in 1963

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An arbovirus serum survey was conducted in March 1963 among residents of various communities within a 50 km radius of Jakarta, Indonesia. The 497 sera collected were analyzed both qualitatively and quantitatively by FCNT for antibodies to Chikungunya, Sindbis, Eastern equine encephalitis, Japanese encephalitis, Yellow Fever, and Bunyamwera viruses. Evidence for the presence of group A, B and Bunyamwera viruses in the areas surveyed is presented. Analysis of the data obtained suggests that Chikungunya, Japanese encephalitis and a virus antigenically related to Bunyamwera were most likely present in most of these communities.

In March of 1963 the U. S. Naval Medical Research Unit No. 2 with the cooperation of Indonesian public health authorities and the Department of Microbiology of the University of Indonesia, School of Medicine collected 497 serum specimens from Indonesians of various ages residing in different areas within a 50 km radius of Jakarta. The sera were analyzed by tissue culture neutralization test for the presence of antibodies to 6 arboviruses including Chikungunya, Sindbis, Eastern equine encephalitis, Japanese B encephalitis, Yellow Fever, and Bunyamwera.

MATERIALS AND METHODS

Antigens used The following tissue culture propagated arboviruses were used in the tissue culture neutralization tests: Chikungunya, Sindbis, Eastern equine encephalitis and Bunyamwera, adapted and propagated in HeLa cells; Japanese B encephalitis (Nakayama strain), yellow fever (17 D strain) and West Nile, adapted and propagated in primary hamster kidney cells.

Preparation of monolayer cell cultures Monolayer cell cultures were prepared utilizing the

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basic techniques of Youngner.[7] Whole kidneys were used for the preparation of hamster kidney (HaK) cultures and were obtained from hamsters weighing 50–100 gms. Trypsinized cells, after washing with maintenance medium to remove the trypsin, were planted in 16×125 mm pyrex screw-capped tubes and incubated at 35°–36°C in a horizontal stationary position. Cell sheets were usually formed in 4–7 days.

HeLa cells (originally obtained from Microbiological Associates) were grown in screw-capped prescription bottles. A versene and trypsin mixture (0.02% of each W/V) in Hanks balanced salt solution was used to remove the cells from the bottles and to prepare cell suspensions for tube cultures. Tubes were inoculated with 50,000–75,000 cells in 1 ml of growth medium and continuous cell sheets formed after 2–3 days' incubation at 35°–36°C.

Media Growth medium for HaK cell cultures consisted of 10% calf serum (CS) and 0.25% lactalbumin hydrolysate (LAH) in Eagle's minimal essential medium (MEM)[1] with the addition of 0.1 mM glycine and 0.1 mM arginine per liter. All media contained 100 units of penicillin and 0.1 mg of streptomycin per ml and a final concentration of 0.002% phenol red. The pH was adjusted to 7.4 by the addition of sterile $NaHCO_3$ solution.

The maintenance medium was the same as the growth medium except that only 5% CS was used.

For the HeLa cell line 5% CS, 0.25% LAH in MEM containing phenol red and antibiotics, as above, were used as both growth and maintenance media.

Tissue culture neutralization tests (TCNT) TCNTs were performed on sera heated for 1/2 hour at 56°C. For qualitative TCNTs a 1:10 dilution of serum was used, and for quantitative TCNTs twofold dilutions of sera starting with a 1:10 dilution were employed. Ten to 100 $TCID_{50}$ of the various viruses were mixed with equal amounts of the appropriate serum dilution, and the serum-virus mixtures were incubated for one hour at room temperature (23°C) prior to the inoculation of 4 tubes with each serum dilution. The inoculated virus dose was re-titrated simultaneously with the TCNT. Following inoculation with the serum-virus mixtures, the tubes were incubated at 35°C and examined daily for cytopathic effect (CPE). When control tubes, inoculated with only the virus dose, showed 75–100% CPE (3–4+) the test was read and the inhibition titers recorded.

RESULTS

Prevalence of antibodies to certain arboviruses in the sera of residents of the Rawasari area of Jakarta, Indonesia Table 1 presents the results of qualitative TCNTs for the detection of antibodies in sera to certain arboviruses from residents of the Rawasari area, a rural community located on the northwest outskirts of Jakarta.

Antibodies to Chikungunya virus were found in 50% of the adult sera tested, while the rate for all ages was 24%. The lower incidence in the younger age groups suggests that contact with this antigen may have been higher in the past and lower since. Infections with antigens of Sindbis virus or those which cross with this virus appear to occur less frequently, as only 26% of the adults contained antibodies with an overall rate of 10%. The results with Eastern equine encephalitis (EEE) virus show only 6% of the adults having antibodies with an overall incidence of 12%. Antibody to a Bunyamwera group virus is prevalent in this area with 38% of the adult sera containing antibody and with an overall incidence of 33%. The 17D strain of yellow fever (YF) virus was neutralized by 68% of the adult sera tested, although only 28% of the sera of all age groups contained antibody. Antibodies to Japanese B encephalitis (JE) virus were detected in only 10% of the adult sera and in 22% of all sera tested. However, the low percentage of adult sera containing antibody suggests that it may not be too prevalent in this area. The JE antibody detected may have been induced by a heterologous group B virus and falls to non-detectable levels in later life. The high incidence of antibodies in adults to YF virus was unexpected, since YF has never been reported in Indonesia. However, it is well known that in

Table 1. Qualitative neutralization* of certain arboviruses by sera of residents, by age group, of the Rawasari Area of Jakarta, Indonesia

Age Group	Group A Arboviruses						Bunyamwera Group		Group B Arboviruses			
	Chikungunya	%	Sindbis	%	Eastern equine Encephalitis	%	Bunyamwera	%	Yellow Fever (17D)	%	Japanese Encephalitis	%
0- 4	1/4‡	25	0/4	0	0/4	0	3/4	75	2/4	50	0/4	0
5- 9	0/34	0	0/34	0	3/34	9	10/34	29	1/34	3	8/34	24
10-14	6/54	11	1/54	2	12/54	22	16/54	30	1/54	2	18/54	33
15-19	3/6	50	1/6	17	0/6	0	1/6	17	4/6	67	2/6	33
⩾20	25/50	50	13/50	26	3/50	6	19/50	38	34/50	68	5/50	10
Total	35/148	24	15/148	10	18/148	12	49/148	33	42/148	28	33/148	22

* Sera tested by TCNT at a 1:10 initial dilution against 10-100 $TCID_{50}$ of known arbovirus. Sera showing inhibition or CPE are considered positive for neutralizing antibodies against the appropriate arboviruses.

‡ Number of sera positive/number of sera tested.

Table 2. Qualitative neutralization* of selected arboviruses by sera of adult residents of the Tanggerang Area (Adult age group: 18-50 years)

Group A Arboviruses						Bunyamwera Group		Group B Arboviruses			
Chikungunya	%	Sindbis	%	Eastern Equine Encephalitis	%	Bunyamwera	%	Yellow Fever (17D)	%	Japanese Encephalitis	%
18/52‡	28	7/69	10	1/69	1	1/69**	1	5/69	7	42/69	61

* Sera tested by TCNT at a 1:10 initial dilution against 10-100 $TCID_{50}$ of known arbovirus. Sera showing inhibition of CPE are considered positive for neutralizing antibodies against the appropriate arboviruses.

‡ Number of sera positive/number of sera tested.

** Previously resided in Central Java.

Table 3. Qualitative neutralization* of certain arboviruses by sera of residents, by age group, of the Tanah Tinggi Area† of Jakarta, Indonesia

Age Group	Group A Arboviruses						Bunyamwera Group		Group B Arboviruses			
	Chikungunya	%	Sindbis	%	Eastern Equine Encephalitis	%	Bunyamwera	%	Yellow Fever (17D)	%	Japanese Encephalitis	%
0– 4	3/22**	14(0)‡	0/22	0	0/22	0	3/22	14(14)	3/22	14(9)	3/22	14(5)
5– 9	2/46	4	2/46	4	4/46	9	2/46	4	5/46	11	5/46	11
10–14	2/22	9	1/22	5	2/22	9	6/22	27	4/22	18	3/22	14
15–19	8/26	31	0/26	0	2/26	8	4/26	15	1/26	4	5/26	19
⩾20	47/93	51	8/93	9	2/93	2	8/93	9	12/93	13	21/93	23
Total	62/209	30(28)	11/209	5	10/209	5	23/209	11	25/209	12(11)	37/209	18(17)

* Sera tested by TCNT at a 1:10 dilution against 10–100 $TCID_{50}$ of known arbovirus. Sera showing inhibition of CPE are considered positive for neutralizing antibodies against the appropriate arboviruses.
† Including 68 patients in the University of Indonesia Hospital.
** Number of sera positive/number of sera tested.
‡ ( )=% positive when sera from children up to 18 months are counted as negative, since Oriental women breast-feed three years or longer and the sera of children through 18 months of age are likely to contain maternal antibody.

Table 4. Qualitative neutralization* of certain arboviruses by sera of residents, by age group, of Tjibinong (a rural area near Bogor) Indonesia

Age Group	Group A Arboviruses						Bunyamwera Group		Group B Arboviruses			
	Chikungunya	%	Sindbis	%	Eastern Equine Encephalitis	%	Bunyamwera	%	Yellow Fever (17D)	%	Japanese Encephalitis	%
5– 9	0/3**	0	1/3	33	1/3	33	0/3	0	0/3	0	0/3	0
10–14	1/5	20	0/5	0	1/5	20	0/5	0	0/5	0	0/5	0
15–19	2/8	25	0/8	0	1/8	13	0/8	0	0/8	0	1/8	13
⩾20	19/55	35	2/55	4	9/55	16	2/55	4	0/55	0	19/55	35
Total	22/71	31	3/71	4	12/71	17	2/71	3	0/71	0	20/71	28

* Sera tested by TCNT at a 1:10 initial dilution against 10–100 $TCID_{50}$ of known arbovirus. Sera showing inhibition of CPE are considered positive for neutralizing antibodies against the appropriate arboviruses.
** Number of sera positive/number of sera tested.

Table 5. Titration range of arboviras neutralizing antibodies from an Indonesian population in 1963

Antigen	Positive serum / Total serum	%	Serum Dilution							
			10[a]	20	40	80	160	320	640	>640
Group A										
Chikungunya	137/497	28	32[b](23)[c]	8(6)	3(2)	5(4)	7(5)	15(11)	12(9)	55(40)
Eastern equine encephalitis	31/497	6	7 (23)	8(26)	9(29)	3(10)	3(10)	1(3)	0	0
Sindbis	25/497	5	23 (92)	1(4)	1(4)	0	0	0	0	0
Group B										
Japanese B encephalitis (Nakayama strain)	121/497	24	14 (11)	13(11)	9(7)	20(17)	9(7)	15(12)	4(3)	37(31)
Yellow fever (17D strain)	52/497	11	28 (54)	13(25)	4(8)	3(6)	2(4)	2(4)	0	0
Bunya wera	67/497	13	32 (48)	17(25)	8(12)	6(9)	2(4)	0	0	1(1)

a—Reciprocal of serum dilution.
b—Number of positive serums.
c—Percentage of positive serums.

areas endemic for dengue and other group B arboviruses, secondary infections with these viruses produce cross-reacting antibodies to other group B viruses, which may explain our findings of a high incidence of antibody to YF virus in adults in this area.

Prevalence of antibodies to certain arboviruses in adult residents of the Tanggerang area of Indonesia Table 2 presents the results of qualitative TCNTs for antibodies in the sera of adult residents of the Tanggerang area, a rural district 25 km west of Jakarta. The incidence of antibodies to Chikungunya, Sindbis and EEE viruses in the sera of adults from this area were 26, 10 and 1%, respectively, much lower than that found in the Rawasari area. Only 1% of those tested had antibodies to Bunyamwera virus in this area and that individual had previously resided in Central Java. Antibodies to JE virus were found in 61% of the adults in Tanggarang while only 7% had antibodies to YF virus. Tanggerang is inhabited predominantly by Indonesians of Chinese ancestry and is a pig-raising area. Since pigs are considered to be the major amplifying host of JE virus, this would explain the higher incidence of antibodies to this virus in this area.

Prevalence of antibodies to certain arboviruses in residents of the Tanah Tinngi area of Indonesia Table 3 presents the results of qualitative TCNTs for arbovirus antibodies in the sera of residents of Tanah Tinngi, an urban area in central Jakarta and includes 68 patients, mostly children, from the University of Indonesia Hospital. In this area the highest incidence of antibodies was found to Chikungunya virus followed by JE, YF, Bunyamwera, Sindbis and EEE. The overall incidence of antibodies to JE virus was about the same as that found in the Rawasari area, but the incidence of antibodies to YF virus was much lower, 12% vs 28%.

Prevalence of antibodies to certain arboviruses in residents of the Tjibinong area of Indonesia Table 4 presents the results of qualitative TCNTs for arbovirus antibodies in the sera of predominantly adult residents of Tjibinong, a rural area near Bogor about 50 km south of Jakarta. Here, too, antibodies to Chikungunya virus appear to be of highest incidence closely followed by JE. A 17% overall incidence of antibodies to EEE virus, the highest incidence found in any of the areas studied, was unanticipated. Few of the sera contained antibodies to Sindbis or Bunyamwera viruses and, surprisingly, none at all to YF virus.

Titration range of arbovirus neutralizing antibodies from an Indonesian population in 1963 Table 5 presents the results of quantitative neutralization tests of sera shown to contain antibodies

Table 6. Arbovirus neutralizing antibody titers from an Indonesian population in 1963 grouped according to low antibody titer (10–40) and high antibody titer (80–>640)

Antigen	Grouped serum neutralizing antibody titers*		
	10–40	80–>640	Totals
Group A			
Chikungunya	43(31%)	94(69%)	137(100%)
Eastern equine encephalitis	24(77%)	7(23%)	31(100%)
Sindbis	25(100%)	0	25(100%)
Group B			
Japanese B encephalitis (Nakayama strain)	36(30%)	85(70%)	121(100%)
Yellow fever (17D strain)	45(87%)	7(13%)	52(100%)
Bunyamwera Group			
Bunyamwera	57(85%)	10(15%)	67(100%)

* Expressed as the reciprocal of the serum dilution.

at the 1:10 level to the 6 arboviruses used in the qualitative tests reported above. Not all sera originally producing neutralization at this level could be tested quantitatively due to insufficient serum remaining, but the numbers lost in this way produced only a 2–4% reduction in the percentage figures obtained by dividing the positive serums by the total number of serums tested, shown in the table without changing the basic trend. Among the group A arboviruses antibodies to Sindbis virus showed the lowest levels with 92% having a titer of only 1:10 whereas 40% of the sera had titers >1:640 to Chikungunya virus. Titers to EEE virus fell between these two extremes. Antibodies to a group B arbovirus such as JE virus were found at high titer (>1:640) in 31% of the sera initially found positive at 1:10 dilution. Titers of antibodies to YF, on the other hand, were generally low with 79% in the 1:10–1:20 range. Sera tested against Bunyamwera virus also showed generally low titers with 73% falling in the 1:10–1:20 range.

Table 6 shows the arbovirus neutralizing antibody titers shown in Table 5 but grouped according to low antibody titer (1:10–1:40) and high antibody titer (1:80–>1:640). It is readily apparent that the great majority of the Indonesian population tested had high antibody titers to only Chikungunya and JE viruses indicating that these or closely related viruses were prevalent in 1963 in an area within a 50 km radius of Jakarta.

DISCUSSION

Little appears to be known of the incidence or kind of arbovirus infections in Indonesia. The analysis of qualitative and quantitative TCNTs for arbovirus antibodies in 497 sera obtained from residents in various communities within a 50 km radius of Jakarta in 1963 has provided definite evidence for the presence of Groups A, B and Bunyamwera-like viruses in this area. The results of this serological survey are starting to be confirmed by the isolation and identification of Japanese encephalitis virus from specimens obtained in the Jakarta area.[6] The finding of neutralizing antibody to Chikungunya and JE viruses in the high titer range (1:80–>1:640) in 70% of all sera tested indicates the presence of these or very closely related viruses in this area. Since only relatively small percentages of sera contained high antibody titers to EEE, YF and Bunyamwera viruses and one of the sera tested with Sindbis virus fell in the high titer range, it may be concluded that the antibody found was induced by related viruses which crossed with the antigens used in the test. Unpublished results from our laboratory of cross-TCNTs with rabbit antisera prepared against JE and the 17D strain of YF showed no crossing between these two viruses and their antisera. However, antibodies to YF virus were found in this survey even though no disease resembling YF occurs in Indonesia; therefore, the presence of such antibody may be the result of primary or secondary infection with other group B aboviruses such as dengue, since it has been recently shown that patients with HI titers of less than 1:80 to YF virus in their acute sera developed HI titers of 1:5120 against YF virus and neutralization titers in the 1:500 range following infection with Dengue 2 virus.[5] Primary or secondary infections with the dengue viruses also may have contributed to high titers obtained against JE virus. These interpretations are supported by the qualitative TCNT results from the Tjibinong area, where none of the sera contained antibody, even at the 1:10 level, against YF virus whereas 35% had antibody against JE virus. This also suggests that infection with the dengue viruses did not occur in this area in 1963 or for a number of years earlier. The sera were not tested against any of the dengue antigens, as CPE was not produced by these viruses in the cell lines available to us.

Hotta's[8] serological surveys of arbovirus in several areas of Indonesia indicated that a small percentage of individuals sampled in Lombok, Lampung and Madjatengka in 1964, 1965 and 1966, respectively, showed HI antibodies against Chikungunya virus. Whereas in 1968 about 40% of the subjects tested in Surabaja contained antibodies to Chikungunya virus ranging in HI titer from 1:20–1:640. Our results by the more specific TCNT show that in 1963 an average of 30% of the population sampled in the Jakarta area contained antibody to Chikungunya virus and of these 65% had titers ranging from 1:160–>1:640 (Table 5). In addition, evidence of the

presence of a virus of the Bunyamwera group was shown by us to be present in the Jakarta area with the greatest prevalence in the Pawasari and the Tanah Tinngi districts.

Since Chikungunya virus has been isolated in Thailand, Malaya, and other Southeast Asian countries,[4] it is most probable that our and Hotta's[2] serological results represent evidence for its presence in Indonesia. The isolation in Kuala Lumpur[5] of Batai virus, one of the Bunyamwera group viruses, and the presence of antibodies to Bunyamwera virus in the Indonesian sera tested, suggest that Batai virus or a virus antigenically related to it is present in Indonesia.

Since it is obvious from these serological studies that groups A, B and Bunyamwera viruses are present in Indonesia, studies should be initiated to attempt to isolate and identify such viruses from men, mosquitoes, natural animal hosts and sentinel animals.

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一九六三年印尼一百公里直徑內之 Arbovirus 血清學調查

美國海軍第二醫學研究所

葛林 赫伯特 余漢民 勤恩 金肯

(62年9月15日受理)

這些 Arbovirus 血清是一九六三年三月在印尼雅加達50公里半徑的範圍內之不同的社區內居民中所收集的資料。

四百九十七個血清都曾利用組織培養中和反應做它們對 Chikungunya, Sindbis, Eastern equine encephalitis, Japanese encephalitis (日本腦炎) Yellow Fever (黃熱病), 以及 Bunyamwera 等病毒的質和量的抗體試驗。

實驗證實在這調查的地區中有 Arbovirus 的 Group A, B 及 Bunyamwera 病毒存在。

從獲得的資料中也可分析並暗示 Chikungunya, Japanese encephalitis 以及在抗原上與 Bunyamwera 病毒相關之病毒也很可能存在這些社區中。